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Central and peripheral integration of interrenal and thyroid axes signals in common carp (*Cyprinus carpio* L.)

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Abstract

In teleostean fishes the hypothalamic–pituitary–thyroid axis (HPT axis) and the hypothalamic–pituitary–interrenal axis (HPI axis) regulate the release of thyroid hormones (THs) and cortisol respectively. Since many actions of both hormones are involved in the regulation of metabolic processes, communication between both signal pathways can be anticipated. In this study, we describe central and peripheral sites for direct interaction between mediators of both neuroendocrine axes in the common carp (*Cyprinus carpio*). Despite suggestions in the literature that CRH is thyrotropic in some fish; we were not able to establish stimulatory effects of CRH on the expression of the pituitary TSH β subunit gene. In preoptic area tissue incubated with 10^{-7} M thyroxine (T_4) a 2.9-fold increase in the expression of CRH-binding protein (CRHBP) was

observed. Thus, T_4 could reduce the bioavailable hypothalamic crh via the up regulation of crhbp expression and hence down regulate the HPI axis. At the peripheral level, cortisol (10^{-6} M), ACTH (10^{-7} M), and α -MSH (10^{-7} M) stimulate the release of T_4 from kidney and head kidney fragments, which contain all functional thyroid follicles in carp, by two- to fourfold. The substantiation of three pituitary thyrotropic factors, viz. TSH, ACTH, and α -MSH, in common carp, allows for an integration of central thyrotropic signals. Clearly, two sites for interaction between the HPT axis, the HPI axis, and α -MSH are present in common carp. These interactions may be key to the proper regulation of general metabolism in this fish.

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Introduction

Thyroid hormones (THs) and corticosteroids are major endocrine signals that are involved in the regulation of fundamental and basic physiological processes in vertebrates. The action of THs is pleiotropic and often permissive, but generally contributes to the regulation of growth, metabolism, development, and metamorphosis (Blanton & Specker 2007). Glucocorticosteroids are considered to be prime stress hormones that govern the stress response through the redistribution of energy toward processes required for coping with the stressor (Wendelaar Bonga 1997). Because of the significance of THs and corticosteroids in the regulation of metabolic processes, we postulate that a bidirectional communication between these endocrine systems is a necessity for the integration and proper functioning of either system.

In teleostean fishes, the THs thyroxine (T_4 , 3,5,3',5'-tetraiodothyronine) and T_3 (3,5,3'-triiodothyronine) are the end products of the hypothalamic–pituitary–thyroid (HPT) axis where hypothalamic TRH stimulates the release of pituitary TSH, which stimulates the release of THs. Similarly, the hypothalamic–pituitary–interrenal (HPI) axis controls the release of cortisol from the interrenal cells in the head kidney, via hypothalamic CRH and pituitary ACTH. The

hypothalamic and pituitary components of both axes are inhibited through negative feedback by their respective end products. The multilevel control of TH and cortisol release allows for potential multiple sites of interaction between both endocrine systems.

Interactions between the HPT and HPI axes have been described in teleostean fishes. Long-term and short-term exposure to cortisol or dexamethasone resulted in decreased levels of plasma THs in several fish species (Redding *et al.* 1984, 1986, Brown *et al.* 1991, Walpita *et al.* 2007). These decreased hormone levels were associated with either increased clearance of plasma THs (Redding *et al.* 1986) or changes in the activity and expression of deiodinases (Walpita *et al.* 2007). Stimulatory effects of cortisol on the HPT axis have also been suggested in teleost fish. In brook charr (*Salvelinus fontinalis*) long-term exposure to cortisol increased the hepatic conversion of T_4 to T_3 (Vijayan *et al.* 1988) and in Japanese flounder (*Paralichthys olivaceus*) cortisol augmented the effects of THs on the resorption of the dorsal fin ray (de Jesus *et al.* 1990). Experimental data on the effects of THs on the HPI axis in teleosts are scarce. In pre- and post-smolt coho salmon (*Oncorhynchus kisutch*), thyroxine treatment resulted in increased and decreased sensitivity of the head kidney to ACTH respectively (Young & Lin 1988).

In several species from all non-mammalian vertebrate classes, CRH does not only stimulate the release of ACTH,

but also that of tsh (De Groef *et al.* 2006). Indeed, in common carp (*Cyprinus carpio*), CRH has been suggested to exhibit thyrotropic activity. In this species, TRH does not stimulate the release of TSH from cultured pituitary cells (Kagabu *et al.* 1998). Moreover, experimental treatment with thyroxine resulted in a marked hypocortisolemia in carp, which was accompanied by an increased mRNA expression of CRH-binding protein (CRHBP) in the preoptic area and unchanged levels of CRH and prepro-TRH mRNA (Geven *et al.* 2006). It appears that, in common carp, not TRH but CRH is controlling the activity of the thyroid gland. Because of its corticotropic and putative thyrotropic activity, CRH neurons may constitute a central site for the communication between the HPT and the HPI axis in common carp.

An investigation on the location of the thyroid gland in common carp revealed another putative site for the integration of the HPT and the HPI axes signals. Whereas in most fishes the thyroid follicles are located in the subpharyngeal region, surrounding the ventral aorta, in common carp all functional thyroid follicles, as characterized by iodine uptake and TSH-mediated T_4 release, are scattered throughout the kidney and head kidney (Geven *et al.* 2007). The close juxtaposition in the head kidney of TH-producing follicles to cortisol-producing interrenal cells strongly hints at a paracrine interaction between both endocrine tissues.

Besides the apparent communication between HPT and HPI axes signals in common carp, a third endocrine signal appears to be involved in these axes. Plasma levels of α -MSH are increased in hyperthyroid and stressed carp (Metz *et al.* 2005, Geven *et al.* 2006), the exact physiological role of which still is unclear. We hypothesize that in common carp the preoptic area and the head kidney represent a central and a peripheral site respectively, for the integration of signals of the HPT and the HPI axis. In this study, we investigated this hypothesis by performing *in vitro* incubations of preoptic area tissues, pituitary glands, and renal tissues with several mediators of both neuroendocrine systems, including α -MSH.

Materials and Methods

Animals

Common carp (*C. carpio*), hereafter called carp, of the all male E4×R3R8 isogenic strain (Bongers *et al.* 1998) were obtained from the Department of Fish Culture and Fisheries of Wageningen University (The Netherlands). Fish were kept in 140-l tanks with aerated, circulating, city of Nijmegen tap water, at a photoperiod of 16h light:8h darkness at 23 °C. Carp were fed Trouvit dry food pellets (Trouw Nutrition International, Putten, The Netherlands) once daily at a ration of 1.5% of the estimated body weight. Before collection of tissues, fish were deeply anesthetized with 0.1% (v/v) 2-phenoxyethanol and killed by spinal transection. Animal handling followed approved university guidelines.

Static incubation of preoptic area and pituitary gland

The pituitary gland and the preoptic area containing the nucleus preopticus (NPO) were dissected as described by Metz *et al.* (2006b). The preoptic areas were diced in $\sim 2 \text{ mm}^3$ sized fragments, while the pituitary glands were kept intact. The quality of dissection was assured by stereomicroscopic analysis. The preoptic area fragments and pituitary glands were immediately transferred to 1 ml ice-cold Leibovitz's L-15 medium (Invitrogen) containing 100 $\mu\text{g/ml}$ kanamycin (Invitrogen), and antibiotic/antimycotic (1×) (Invitrogen). After 1 h, the preoptic area fragments were carefully transferred to 1 ml fresh L-15 medium supplemented with T_4 (at concentrations of 10^{-8} and 10^{-7} M respectively), while the pituitary glands were transferred to 1 ml fresh L-15 medium supplemented with T_4 (10^{-8} and 10^{-7} M), ovine (O) CRH (10^{-7} M), or human TRH (10^{-7} M). L-15 medium of controls did not receive any supplement. Thyroxine, oCRH, and hTRH were obtained from Sigma Chemical Co.

Preoptic area fragments and the pituitary glands were incubated for periods as indicated in the legends to the figures. All tissues were incubated at 22 °C, while continuously shaking (200 r.p.m). Each medium was replaced at 1, 6, 12, and 24 h after the start of incubation. After the incubation, the preoptic area fragments and pituitary glands were immediately stored at -80°C until further processing.

RNA extraction and cDNA synthesis

To extract total RNA, the preoptic area fragments and the pituitary glands were homogenized in 500 μl TRIzol reagent (Invitrogen) by ultrasonification. Following treatment with DNase, 1 μg RNA was reverse transcribed to cDNA in a 20 μl reaction mixture containing 300 ng random primers, 0.5 mM dNTPs, 10 mM dithiothreitol, 10 U RNase Inhibitor, and 200 U Superscript II Reverse Transcriptase (Invitrogen) for 50 min at 37 °C and stored at -20°C .

Real-time quantitative PCR

Since homologous antibodies against the peptides we wished to quantify are not available for carp, and heterologous antibodies are validated for qualitative purposes only, we measured the expression of *crh*, prepro-trh (*pp-trh*), urotensin I (*uts1*), and *crhbp* mRNA in the preoptic area and of TSH β (*tshb*) subunit, proopiomelanocortin (*pomc*), proprotein convertase subtilisin/kexin type 1 (*psk1*), and prolactin (*prl*) mRNA in pituitary gland by real-time quantitative PCR (RQ-PCR). In general, cDNA was diluted ten times, but a portion of pituitary gland cDNA was diluted 500 times to measure *pomc* gene expression. Totally 5 μl cDNA was used in a 25 μl reaction mixture consisting of 12.5 μl Sybr Green Master Mix (PE Applied Biosystems Benelux, Nieuwerkerk aan den IJssel, The Netherlands), and 3 μl of each primer (600 nM final concentration). The primer sets used for PCR are shown in Table 1. The RQ-PCR was performed on a

Table 1 Primer sequences with corresponding GenBank accession numbers. Open reading frame positions are relative to the start codon

	Accession number	Primer	Open reading frame position	Sequence 5' → 3'
Gene product				
crh	AJ317955	qcrh-fw	162–181	CATCCGGCTCGGTAACAGAA
		qcrh-rv	277–257	CCAACAGACGCTGCGTTAACT
pp-trh	AB179818	qtrh-fw	950–969	TTACCGGTGCGTTTCACGTT
		qtrh-rv	1079–1060	AGCTGTGTGCCAAACCAAAAC
uts1	M11671	quts1-fw	-15–5	GCACCTGTGTCCAGCATGAA
		quts1-rv	69–52	GGTGCTCAGCGGGATGTG
crhbp	AJ490880	qcrhbp-fw	680–701	ACAATGATCTCAAGCGGTCCAT
		qcrhbp-rv	746–727	CCACCCAGAAGCTCGACAAA
tshb	AB003585	qtshb-fw	230–249	AGTACCGAAGCGGCCATTGT
		qtshb-rv	297–278	TGCCACCGGATAGGTGAAGT
pomc	Y14618	qpomc-fw	40–60	TTGGCTCTGGCTGTCTGTGT
		qpomc-rv	176–152	TCATCTGTGATCAGACCTGCATA
pcsk1	AM236095	qpcsk1-fw	1300–1319	GCTGGTTTGATGGTCAACAG
		qpcsk1-rv	1381–1361	GACTTTGGGATCAGCCAGAT
β-actin	M24113	qact-fw	342–366	CAACAGGGAAAAGATGACACAGATC
		qact-rv	424–406	GGGACAGCACAGCCTGGAT
40S	AB012087	q40S-fw	365–384	CCGTGGGTGACATCGTTACA
		q40S-rv	433–410	TCAGGACATTGAACCTCACTGTCT

GeneAmp 5700 Sequence Detection System (PE Applied Biosystems). The reaction mixture was incubated for 10 min at 95 °C, followed by 40 cycles of 15 s denaturation at 95 °C and 1 min annealing and extension at 60 °C. Analysis of dissociation plots confirmed the specificity of the PCRs. Cycle threshold values were determined from amplification curves. The expression of genes of interest was calculated relative to 40S ribosomal protein S11 mRNA expression.

Static incubation of kidney and head kidney

Head kidneys and kidney tissue were removed from the animal, diced into ~ 2 mm³ sized fragments and immediately placed in appropriate volume of ice-cold Leibovitz's L-15 medium (Invitrogen) containing 100 µg/ml kanamycin (Invitrogen) and antibiotic/antimycotic (1×) (Invitrogen). After 1 h, the head kidney and kidney fragments were carefully transferred to 2 ml fresh L-15 medium supplemented with cortisol (at concentrations of 10⁻⁷ and 10⁻⁶ M respectively) human ACTH (10⁻⁷ M) or monoacetyl α-MSH (10⁻⁷ M), while control incubations did not receive any supplement. Cortisol, hACTH, and α-MSH were from Sigma Chemical Co.

Tissues were incubated for 24 h at 22 °C, while continuously shaking (200 r.p.m.), after which the 2 ml incubation medium was separated from the tissue by centrifugation (4 °C, 1000 g, 15 min) and reduced to 0.5 ml by vacuum drying. The incubation medium was applied to a Sephadex LH-20 column to isolate the THs and to remove salts (Mol & Visser 1985). In short, glass pipettes were filled with 1 ml Sephadex LH-20 (Amersham Biosciences) suspension in water (10% w/v) and equilibrated with 3 volumes of 1 ml 0.1 M HCl. Samples were acidified with an equal volume of 1 M HCl and loaded on to the column. The samples were then eluted from the column with 5 volumes of 1 ml 0.1 M HCl for the removal of ions, 4 volumes of 1 ml H₂O to neutralize the column, and 3 volumes of 1 ml

0.1 M NH₃/EtOH to collect THs. The fractions containing THs were vacuum dried and reconstituted in 60 µl 50 mM sodium barbitone/0.1% BSA buffer (pH 8.6). Total thyroxine was measured in duplicate with a total T₄ ELISA (Human Gesellschaft für Biochemica und Diagnostica GmbH, Wiesbaden, Germany) according to the manufacturer's instruction. Standards were prepared in the same barbitone buffer as the samples were. The intra-assay and inter-assay coefficients of variation for the tT₄ ELISA reported by the manufacturer are 4.2 and 3.3% respectively. The reported cross reactivity of the ovine anti-T₄ antibody to D-T₄ is 98% (the reactivity to L-T₄ is set at 100% as a reference), and to L-T₃ and D-T₃ is 3 and 1.5% respectively. Cross-reactivities of the antibody to diiodothyronine, diiodotyrosine, and iodotyrosine are <0.01%. The reported sensitivity of the tT₄ ELISA is 4 ng/ml T₄. Protein content of the incubated tissues was determined by Bio-Rad protein assay (Bio-Rad) using BSA as reference.

Statistical analysis

All data are represented as mean values ± s.d. The number of different preparations (*n*) is given in parentheses. Differences between groups were assessed with Student's parametric *t*-test for unpaired observations, or Mann–Whitney's non-parametric *U*-test, where appropriate. Statistical significance was accepted at *P* < 0.05 (two-tailed), probabilities are indicated by asterisks (*, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001).

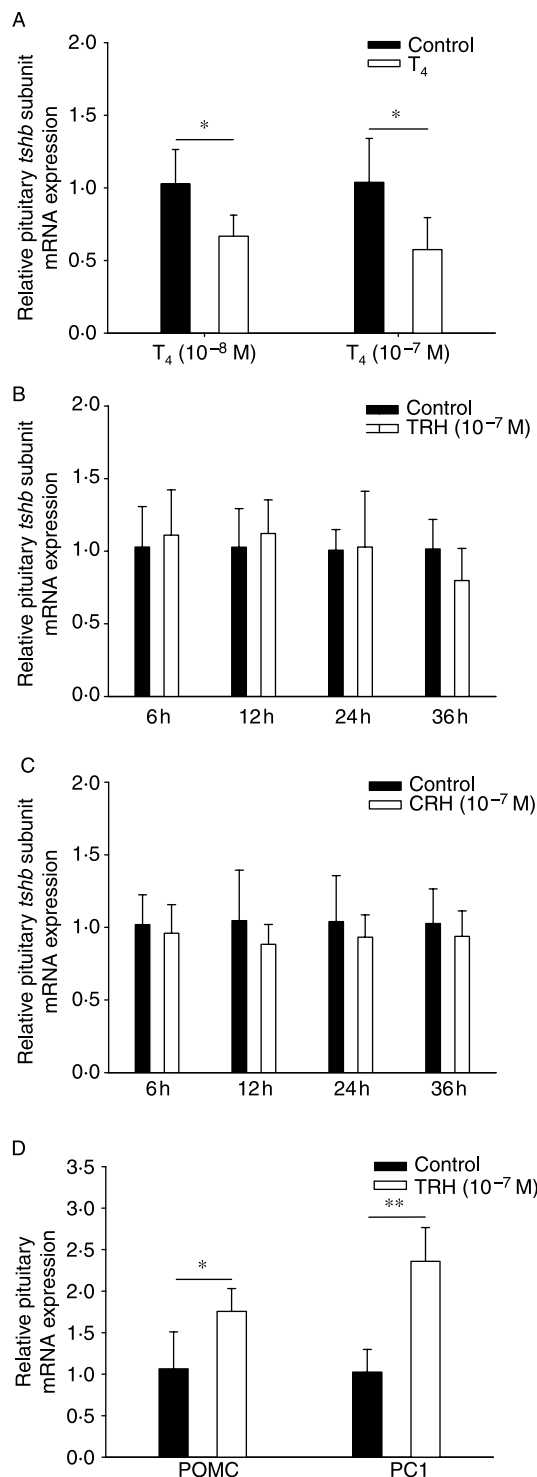
Results

Effects of CRH and TRH on pituitary tshb gene expression

Thyroxine, at concentrations of 10⁻⁸ and 10⁻⁷ M, significantly down regulated the expression of *tshb* subunit mRNA

by 35% ($P=0.01$) and 45% ($P=0.02$) compared with control incubations respectively (Fig. 1A), which demonstrated the viability of the pituitary gland preparation. Neither TRH (10^{-7} M) nor CRH (10^{-7} M) altered *tshb* subunit gene

expression (Fig. 1B and C). Incubation of pituitary glands with TRH (10^{-7} M) increased the expression of *pomc* ($P=0.04$) and *pcsk1* ($P=0.002$) 1.7- and 2.3-fold respectively (Fig. 2D), which confirmed the bioactivity of TRH. The integrity and bioactivity of the CRH preparation used was confirmed in our laboratory by mass spectrometry and the stimulatory action on the release of ACTH and α -MSH from carp pituitary glands *in vitro* (Metz *et al.* 2004, van den Burg *et al.* 2005).



Effects of T_4 on gene expression in the preoptic area

Thyroxine, at 10^{-7} M, significantly increased the expression of *crh*, *prepro-trh*, and *crhbp* in the preoptic area 4.6- ($P=0.0001$), 2.9- ($P=0.002$), and 2.1-fold ($P=0.04$) respectively (Fig. 2). The expression of *uts1* remained unchanged (Fig. 2D). Incubation with 10^{-8} M T_4 had no statistically significant effects on the expression of any of the genes tested (Fig. 2).

Effects of cortisol, ACTH and α -MSH on the release of T_4 from renal tissues

Cortisol at 10^{-6} M, but not at 10^{-7} M, stimulated the release of T_4 from head kidney ($P=0.04$) and kidney tissue ($P=0.01$) 3.5-fold (Fig. 3). ACTH and α -MSH (both at 10^{-7} M) increased the release of T_4 from head kidney and kidney tissue two- to four-fold (Fig. 3). Basal and stimulated T_4 -secretion from the head kidney, overall, was ten times lower than that of the kidney (Fig. 3).

Discussion

We demonstrate here that the NPO and renal tissues are putative sites for interaction between mediators of the HPT- and the HPI axis in carp. Thyroxine affects the CRH system in the preoptic area. Peripherally, cortisol and ACTH both stimulate the release of THs from renal tissues, viz. the head kidney and kidney.

Despite indications that preoptic CRH may be involved in the regulation of the HPT axis in some fish (Kagabu *et al.* 1998, Geven *et al.* 2006, De Groef *et al.* 2006), no effect of CRH on the expression of carp pituitary *tshb* subunit could be demonstrated *in vitro*. The absence of a thyrotropic action of preoptic CRH can also be inferred from experimental results obtained *in vivo*. When carp were exposed to a 24 h confinement stressor, the HPI axis was markedly activated, as exemplified by an increased expression of *crh* mRNA in the preoptic area (Huising *et al.* 2004). However, this increase was not accompanied by increased expression of pituitary *tshb*

Figure 1 Relative mRNA expression levels of pituitary *tshb* subunit upon (A) 36 h incubation with T_4 (10^{-8} M, $n=6$ and 10^{-7} M, $n=5$) and (B) 6, 12, 24, and 36 h incubation with TRH (10^{-7} M, $n=4$) and (C) CRH (10^{-7} M, $n=6$). Relative mRNA expression of pituitary *pomc* and *pcsk1* upon (D) 6 h incubation with TRH (10^{-7} M, $n=4$).

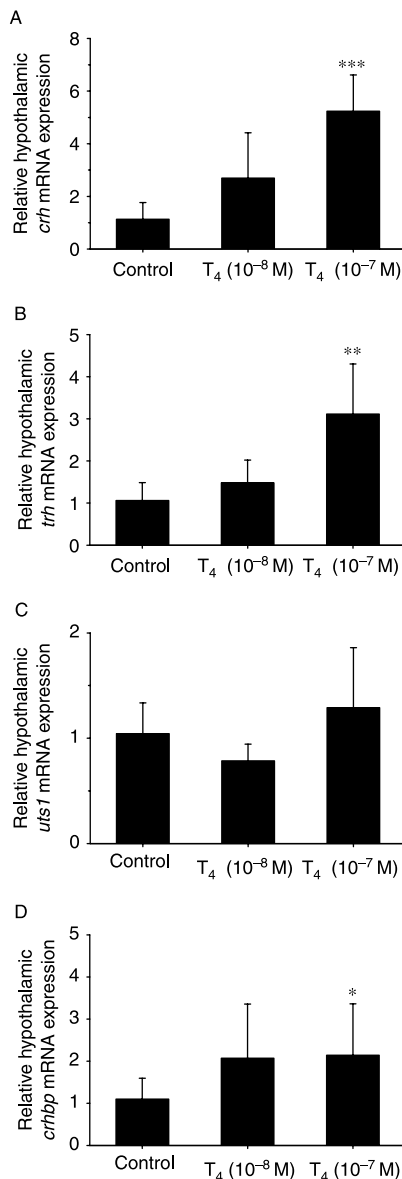


Figure 2 Relative mRNA expression levels of (A) *crh*, (B) *prepro-trh*, (C) *uts1* and (D) *crhbp* (D) in hypothalamic tissue ($n=6$) incubated for 36 h with 10^{-8} M and 10^{-7} M T_4 .

subunit, which remained unaffected in these animals (Dr J R Metz, personal communication). Similarly, TRH did not alter the expression of *tshb* subunit gene *in vitro*, corroborating the results of Kagabu *et al.* (1998). Taken together, these data provide no evidence for CRH and TRH as a hypothalamic thyrotropic factor in carp. However, the thyroid-stimulating properties of ACTH and α -MSH in carp may still confer a thyrotropic action to CRH and TRH. The present study focused on TSH as the pituitary thyroid-stimulating factor in carp (Geven *et al.* 2006), but the identification of ACTH and α -MSH as two new putative pituitary thyroid-stimulating

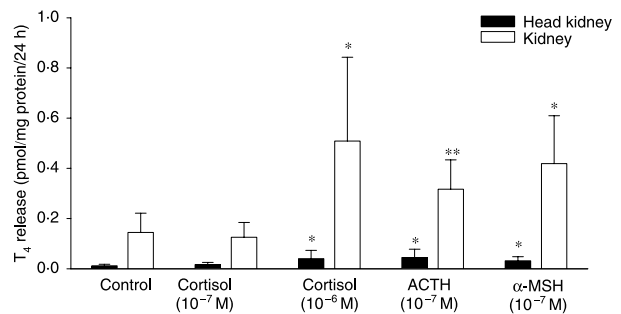


Figure 3 Release of T_4 from head kidney and kidney tissue ($n=6-7$) incubated for 24 h with cortisol (10^{-7} and 10^{-6} M), ACTH (10^{-7} M) and α -MSH (10^{-7} M, $n=3$).

factors re-establishes CRH and TRH as potential hypothalamic thyrotropic factors, since the release of ACTH and α -MSH in carp is stimulated by CRH and TRH (van den Burg *et al.* 2003, 2005, Metz *et al.* 2004). The identification of more than one hypothalamic thyrotropic and pituitary thyroid-stimulating factor in carp, clearly points to an integration of multiple endocrine signals for the control of the thyroid gland activity in teleostean fishes.

THs can modulate, at a central level, the HPI axis in carp. The expression of preoptic *crh* mRNA is markedly increased upon exposure to T_4 *in vitro*, although this effect could not be measured *in vivo* in hyperthyroid carp that were treated with T_4 . Here, the repeated injection of T_4 produced a pronounced down regulation of the HPI axis as evidenced by a decreased level of plasma cortisol. The expression of preoptic *crh* mRNA however, remained unaffected (Geven *et al.* 2006). *In situ*, the preoptic CRH neuron is controlled by a multitude of stimulatory and inhibitory signals (Itoi *et al.* 1998, Pisarska *et al.* 2001). The different expression of *crh* upon T_4 exposure *in vitro* and *in vivo* which we observed can be explained by the fact that the denervated preoptic area *in vitro* does not receive efferent inhibitory signals, viz. glucocorticoids, norepinephrine, GABA, β -endorphin, dynorphin, somatostatin, galanin, and substance P (ibid). Our results indicate that the control of the preoptic CRH neurons by T_4 is modulated by other factors.

A consistent effect of T_4 on the CRH system in carp is the stimulation of the expression of *crhbp* mRNA *in vitro* as well as in hyperthyroid carp *in vivo* (Geven *et al.* 2006). Hypothalamic CRHBP binds CRH (and *uts1*) with a higher affinity than the type 1 *crh* receptor, which reduces the bioavailability of CRH and, subsequently, the CRH-induced release of pituitary ACTH (Potter *et al.* 1991, Cortright *et al.* 1995, Westphal & Seasholtz 2006). Also in carp CRHBP appears to be a functional modulator of hypophysiotropic CRH, since the expression of preoptic area *crhbp* mRNA is elevated upon a 24 h restraint stressor and CRHBP is colocalized in CRH immunoreactive neurons projecting from the hypothalamus to pituitary corticotropes (Huisin *et al.* 2004). The extent to which the T_4 -induced increase in *crhbp* mRNA expression levels *in vitro* translates into increased

functional protein concentrations in the hypothalamus *in situ* is difficult to estimate and awaits the development of a quantitative assay for CRHBP protein. Still, our data suggests that CRHBP from the preoptic area, where expression is T_4 -sensitive *in vitro* as well as *in vivo*, may fulfill a role as a central messenger that allows for the interaction of the HPT axis with the HPI axis in carp.

We also identified a peripheral site for the interaction between the HPT and the HPI axes: the head kidney and kidney. We already established that short- and long-term incubation with THs have no effect on the release of cortisol from head kidney fragments (Geven *et al.* 2006). Conversely, exposure of head kidney and kidney fragments to cortisol and ACTH stimulated the release of T_4 from these tissues. The TH-releasing properties of cortisol and ACTH are consistent with the expression of their specific receptors, i.e. the glucocorticoid receptor and the type 2 melanocortin receptor respectively, in head kidney as well as kidney (Metz *et al.* 2005, Stolte *et al.* 2008).

Although cortisol has been shown to stimulate iodide uptake and thyroglobulin synthesis in synergy with TSH in several mammalian thyroid cell cultures (Roger & Dumont 1983, Gérard *et al.* 1989, Becks *et al.* 1992, Takiyama *et al.* 1994), we describe here a direct and independent effect of cortisol on the thyroid gland of a teleost. Since the kidney is devoid of interrenal cells and a local cortisol-mediated effect therefore is not possible, we conclude that ACTH has a direct effect on the release of T_4 in the kidney. Such a direct effect of ACTH could also apply to the head kidney. However, the ACTH-induced release of T_4 may also represent a paracrine effect of endogenous cortisol released upon stimulation by ACTH, as exogenous cortisol mimicked the effect of ACTH on the release of T_4 from head kidney tissue. Studies into the cellular localization of the type 2 melanocortin receptor in head kidney tissue may reveal the presence of this receptor in thyrocytes, and thus can provide evidence for a direct mode of action of ACTH.

Another novel finding of this study is that the pituitary POMC-derived hormone α -MSH also has thyroid-stimulating properties in carp. The effect of α -MSH in the kidney is consistent with the expression of type 5 melanocortin receptor (Metz *et al.* 2005). Although the MC5R is not expressed in the head kidney of carp, the thyroid-stimulating effect of α -MSH may be mediated by other melanocortin receptors, for instance, the expression of the type 4 melanocortin receptor has been reported in the head kidney of rainbow trout and Japanese pufferfish (*Takifugu rubripes*; Haitina *et al.* 2004, Klovins *et al.* 2004). In rat, binding of a specific analog for α -MSH was observed in the thyroid gland, indicating a regulatory role for α -MSH on thyroid gland metabolism in mammals (Tatro & Reichlin 1987).

We have found that THs stimulate the release of α -MSH in carp *in vivo*. Hyperthyroid carp have plasma levels of α -MSH that are increased by 30%, which are accompanied by increased mRNA expression levels of pituitary pars distalis *pomc* and *psk1* (Geven *et al.* 2006). In teleost fish, including

carp, the release of pituitary α -MSH is mainly attributed to TRH (Lamers *et al.* 1994, van den Burg *et al.* 2003, 2005). The *in vitro* stimulation of *trh* expression by T_4 , and the *in vitro* stimulation of *pomc* and *psk1* expression by TRH observed in this study, is commensurate to the endocrine cascade by which THs control the release of α -MSH in carp.

The widespread distribution of melanocortin receptors in fish illustrates the pleiotropic functions for α -MSH, which includes the regulation of food intake and metabolism (Metz *et al.* 2006a). The intracerebroventricular injection of [Nle⁴, α -Phe⁷]- α -MSH, an α -MSH agonist, inhibited food intake in goldfish (*Carassius auratus*; Cerdá-Reverter *et al.* 2003) and peripherally, α -MSH exhibited lipolytic effects in hepatocytes of rainbow trout (*Oncorhynchus mykiss*; Yada *et al.* 2000, 2002). The concerted actions of THs, cortisol, and α -MSH on the regulation of metabolic processes may form the basis for the integration of these signals in carp.

In conclusion, this study identifies a central and a peripheral site in carp for the communication between the interrenal axis and the thyroid axis and, additionally, α -MSH as a thyroid-stimulating factor. Centrally, T_4 can inhibit the HPI axis via CRHBP in the preoptic area. Peripherally, cortisol and ACTH stimulate the release of T_4 from renal tissues, as does α -MSH. The intimate interrelationships between these neuroendocrine systems are pivotal for the regulation of general metabolism.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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